Immediate Effects of Ionizing Radiation on the Structure of Unfrozen Bovine Muscle Tissue

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SUMMARY

To measure the immediate changes in the ultrastructure of meat induced by ionizing radiation, bovine longissimus dorsi *muscle was irradiated with* ^{137}Cs *72 h after slaughter. The temperature of the samples during irradiation was maintained between 0 and 4°C to avoid cellular changes caused by freeze/thaw. Muscle was irradiated at 5, 10, 30 and 50 kGy. At low levels (up to 10 kGy) of radiation, changes in muscle structure were minimal. At levels above 30 kGy major increases in myofibril fragmentation and decreases in the tensile strength of raw and cooked muscles were noted. SDS gel electrophoresis indicated a decrease in myosin. No changes in the ultrastructure were observed using transmission electron microscopy.*

INTRODUCTION

The use of ionizing radiation as a means to preserve meat has been under investigation since the late 1940s. Initial studies concentrated on the effects of large doses (> 10 kGy) on the texture, odor, color (Bratzer & Doty, 1955; Kirn *et al.*, 1956), and residual proteolytic activity (Doty & Wachter, 1955; Drake *et al.*, 1957) in fresh and cooked meat. These and subsequent studies pointed out that the irradiation of food can be beneficial by eliminating or reducing pathogenic microorganisms and food spoiling bacteria, thereby

extending the normal shelf life of some perishable foods. Irradiation can cause some undesirable changes in muscle tissue, but these can be eliminated, or at least minimized, by judicious control of dosage, temperature, packaging and packaging atmosphere. The effect of irradiation on color and flavor (Hanson *et al.*, 1964), nutrients (Josephson *et al.*, 1978), and texture (Segars *et al.*, 1981) has been studied. While irradiation will successfully prolong the shelf life of fresh meat for short storage periods, exposure of uncooked canned meat to sterilizing doses (>20 kGy) of radiation has been associated with structural deterioration after prolonged storage at room or refrigerated temperatures. This loss in texture is attributed to the action of proteinases which are still partially active even when meat is irradiated up to 400 kGy (Rhodes, 1969).

The objective of this study was to determine whether ionizing radiation in the range of 5 to 50 kGy causes any immediate and measurable changes in the muscle structure prior to those induced by the onset of the normally occurring proteolysis.

MATERIALS AND METHODS

Materials

Intact beef ribs were obtained from 1–1/2-year old animals which had been slaughtered at a local abattoir approximately 72 h prior to the initiation of the study. *Longissimus dorsi* (LD) muscle was excised, and all superficial connective and adipose tissue was removed. To obtain homogeneous sampling, the muscle was cut into cubes, mixed, and then randomly selected for irradiation or to serve as controls.

Irradiation

Samples were placed in hermetically sealed low oxygen permeable (1·0 ml/100 in^2/24 h) evacuated plastic pouches. They were then placed in an ice-water bath and irradiated with a ^{137}Cs source (dose rate 0·13 kGy/min) to levels of 5, 10, 30 and 50 kGy. The temperature of the samples was maintained between 0–4°C during the irradiation, rather than below 0°C.

Myofibril fragmentation

To determine myofibril fragmentation, LD muscle was ground through a 4·8 mm plate prior to packaging and subsequent irradiation. Six replicates were run at each dosage level. Ten grams of the ground samples were

blended in a Virtis '45' homogenizer in 50 ml of a chilled (2°C) isolating medium (100 mM KCl, 20 mM K_3PO_4, 1 mM EDTA, 1 mM $MgCl_2$ and 1 mM NaN_3 (Culler *et al.*, 1978) for 1 min at medium speed. The homogenate was filtered through a single layer of cheesecloth and left to drain for 30 min. The filtrate was transferred to preweighed centrifuge tubes and centrifuged in a Sorvall RC-5B refrigerated centrifuge (0–4°C) at $4000 \times g$ for 15 min. The supernatant was decanted; lipid deposits adhering to the sides were removed by wiping, leaving the myofibrillar residue behind. Centrifuge tubes were inverted, drained of excess moisture for 30 min, and then reweighed.

Protein concentration was determined by diluting an aliquot of the myofibrillar suspension and carrying out the biuret test (Gornall *et al.*, 1949).

Tensile strength

Muscle was sliced in a manner so that orientation of the muscle fibers ran parallel to the long axis. To obtain uniform 2 mm thick sections, a Thomas–Stadie–Riggs (Arthur H. Thomas, Philadelphia, PA) tissue slicing device was modified to prepare 7 cm × 3 cm × 0·2-cm thick slices. A die (Din 53 504 75, S 3A, MS Instrument Co., Castleton on Hudson, NY) was used to stamp out dumbbell-shaped sections (5·8 cm × 0·8 cm) from the 0·2 cm thick slices. The sections were weighed and placed in the load cell (Instron Universal Testing Instrument, model 1122, Instron Engineering Corp., Canton, MA) with the muscle fibers oriented parallel to the direction of the applied force. The length of the muscle between clamps was 3·5 cm, and the width of the muscle at its narrowest was 0·4 cm. Tissue breakage at the clamp sites during testing was reduced by covering one serrated surface of each clamp with copper foil. Prior to testing, the tissue was kept in a closed bag immersed in ice water to prevent dehydration. The Instron was calibrated and operated as follows: full scale deflection, 100 g; crosshead speed, 20 mm/min; chart speed, 20 mm/min. Ten replicates were carried out at each dosage level, whenever possible.

Warner–Bratzler Shear

Cores of LD muscle, 21 mm in diameter, were obtained using a cork borer. These samples were cut with the muscle bundles oriented parallel to the long axis of the cylindrical sample. The cores were placed into individual test tubes and heated in a thermostatically controlled water bath (63°C) to an internal temperature of 60°C. Shear measurements were conducted using the shear bars from a Chatillon model SD-50 Warner–Bratzler (WB) shear apparatus, with a WB shear plate having 60° 'V' notch adapted for use in an Instron 1122 Testing Apparatus. The shear bars were mounted on a base

which was placed on a compression load cell (100 kg max) calibrated for full scale deflection at 20 kg. The cooked samples were placed between the base and the 'V' notched shear plate which was clamped in the vise jaws of the Instron. The shear plate was forced through the sample (perpendicular to the fibers) with a crosshead speed of 5 mm/min. The maximum force required to sever the sample was recorded on a chart operating at 20 mm/min.

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

The myofibrillar fraction from 4 g of bovine LD muscle was isolated in the manner described by Culler *et al.* (1978). The temperature of the samples during the preparation was maintained by immersion in an ice bath. Aliquots of the myofibrillar extract and protein standards (MW 17 000–200 000) were heated separately in a boiling-water bath after the addition of SDS and 2-mercaptoethanol. Following the addition of glycerol and bromophenol blue, the samples were subjected to electrophoresis (Laemmli, 1970) on rods (7 mm I.D. × 125 mm) using a 20% crosslinked stacking gel and a 10% acrylamide separating gel. Electrophoresis was carried out at pH 8·3 in a 0·025M tris buffer at a constant current of 1 mA/tube during the migration through the stacking gel and increased to 3 mA in the separating gels. Electrophoresis was stopped after the bromophenol blue tracking dye had traveled approximately 9 cm through the separating gel. The gels were stained overnight with Coomassie brilliant blue R250 containing 1% copper chloride (Williams & Gratzer, 1971). After destaining, the gels were scanned and the bands quantitated using a Shimadzu dual wavelength TLC scanner operating in the transmission mode with a data recorder (DR-2).* Absorbance was measured at 556 nm.

Electron microscopy

To prevent contraction of the tissue, LD muscle was clamped *in situ*, excised, and fixed in 3% glutaraldehyde and 1% paraformaldehyde in 0·05M cacodylate buffer at pH 5·6. The samples were cut into approximately 1 mm cubes after the initial fixation. After washing in the buffer, the samples were postfixed in 1% osmium tetroxide in the same buffer and at the same pH. Following washings with water (3 ×), the samples were dehydrated through an increasing acetone series at ice water temperature and embedded in Spurr resin (Spurr, 1969). Ultra-thin sections were obtained with an L.K.B. IV microtome. Samples were examined with a Zeiss 10-B electron microscope at 60 kv.

* Reference to brand or firm name does not constitute endorsement by the US Department of Agriculture over others of a similar nature not mentioned.

RESULTS AND DISCUSSION

Measurement of myofibrillar fragmentation has been used as an index to predict the tenderness of raw meat, and, when used in conjunction with shear testing, it gives an adequate indication of textural properties. We measured myofibrillar fragmentations within 15 h after irradiation, and before any substantial protease induced breakdown could occur. Myofibrils were isolated to determine whether irradiation caused changes in protein structure which ultimately could affect myofibrillar fragmentation or caused changes in the distribution of the myofibrillar proteins. The procedure described by Reagan *et al.* (1975) was adopted.

The effects of irradiation on myofibrillar fragmentation as measured by the previously described procedure are presented in Table 1. Analysis of

TABLE 1
Effects of Irradiation of Myofibril Fragmentation

Experiment	*Sediment (g)*											
	Control			*5 kGy*			*10 kGy*			*30 kGy*			*50 kGy*		
	n	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*
1	6	3·57	(0·30)				6	4·83	(0·25)	6	5·35	(0·19)	6	5·21	(0·24)
2	5	3·52	(0·44)	4	5·05	(0·22)	5	5·16	(0·30)				5	5·60	(0·79)
3	5	4·63	(0·22)	5	5·88	(0·70)	4	5·81	(0·33)				5	6·12	(0·50)
4	4	3·22	(0·35)	4	3·72	(0·31)	4	4·89	(0·24)	4	6·23	(0·29)	3	7·07	(0·32)
5	4	3·28	(0·45)	5	3·85	(0·88)	4	5·51	(0·28)	5	4·61	(0·30)	4	5·53	(0·58)
Average		3·64			4·63			5·24			5·40			5·91	

n = Number of determinations.

variance (ANOVA) indicates a significant ($p < 0{\cdot}01$) increase in the weight of the myofibrils in the filtrate with increasing dosage, suggesting that irradiation enhances fragmentation of the myofibrils.

To determine changes in the composition of the myofibrillar proteins due to irradiation, the tissue extracts were solubilized, and separated by SDS polyacrylamide gel electrophoresis (Figs 1–3). Spectrophotometric scanning of the gels at 556 nm revealed minor reductions in the areas of the bands at the lower doses. At 50 kGy (Fig. 3) there was a 40% reduction in the area for myosin ($\sim$200 000 daltons) relative to the other bands. Taub *et al.* (1979) have referred to an unpublished study where myosin was heated, frozen, and then irradiated prior to gel electrophoresis. A loss of the high molecular weight protein ($\sim$210 000 daltons) after irradiation (−40°C) at 40 kGy and

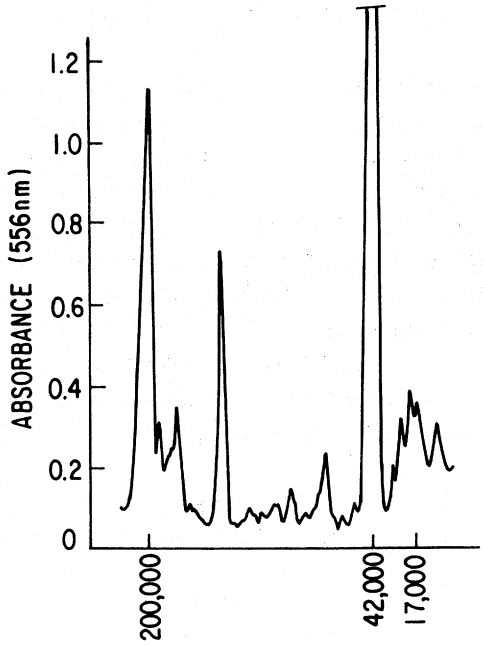

Fig. 1. Densitometric scan of SDS gel electrophoretic separation of myofibrillar protein extract from LD muscle—control.

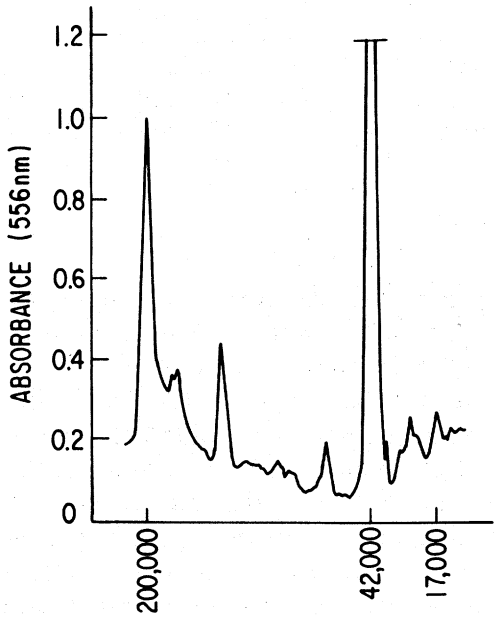

Fig. 2. Densitometric scan of SDS gel electrophoretic separation of myofibrillar protein extract from irradiated LD muscle—30 kGy.

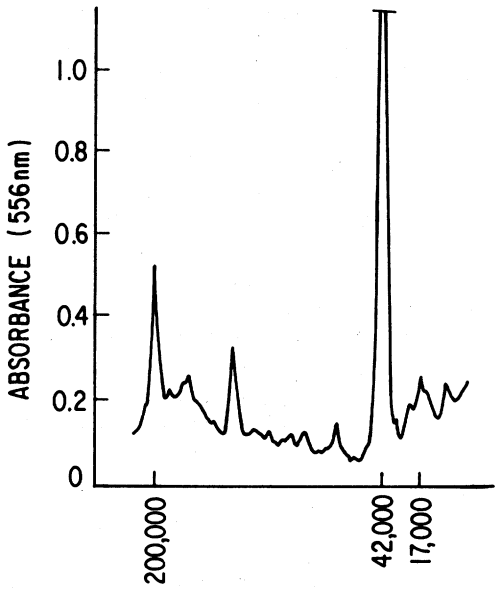

Fig. 3. Densitometric scan of SDS gel electrophoretic separation of myofibrillar protein extract from irradiated LD muscle—50 kGy.

80 kGy was observed in the readily dissolved material. Klein & Altmann (1972) irradiated (~0°C) frozen chicken muscle at levels up to 50 kGy and found it difficult to detect any effects due to irradiation below 5 kGy; however, at sterilizing levels there were apparent depletions in the protein bands between 100 000 and 160 000 daltons. Zabielski *et al.* (1984) noted that irradiation of chicken muscle resulted in a pronounced reduction in the heavy chain myosin (200 000 D), and in the light myosin fraction (15 000–25 000 D). We did not observe the latter.

Tensile strength measurements on raw muscle are presented in Table 2. ANOVA with orthogonal polynomial contrasts shows a significant ($p < 0{\cdot}01$) decrease in tensile strength with increasing exposure to radiation. This is readily apparent at the 30 and 50 kGy levels. Use of the Thomas-Stadie-Riggs tissue slicing device did not always result in samples of equal thickness. Samples which differed greatly in thickness from the intended 2 mm were discarded prior to analysis. Due to the variability usually encountered when conducting physical testing, we attempted to analyze ten samples at each dosage level from different sites within the muscle. The observation of a decrease in the tensile strength of raw muscle after irradiation is in general agreement with hedonic studies conducted on cooked meat.

Shear test data on cooked LD muscle which was irradiated while in the raw state is presented in Table 3. ANOVA with orthogonal polynomial

TABLE 2
Effects of Irradiation on Tensile Strength of Raw LD Muscle

Experiment	*Breaking force (g)*														
	Control			*5 kGy*			*10 kGy*			*30 kGy*			*50 kGy*		
	n	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*	*n*	*Mean*	*SD*
1	8	37·8	(8·8)				10	33·1	(8·0)	12	25·6	(4·7)	5	27·0	(3·4)
2	10	31·3	(7·1)	10	33·8	(18·7)	11	36·9	(10·8)				10	35·9	(6·7)
3	11	26·3	(7·3)	11	59·4	(18·0)	9	37·9	(12·0)				9	25·9	(6·9)
4	11	81·5	(27·3)	9	61·4	(18·2)	10	39·2	(6·4)	10	27·2	(7·6)	10	21·8	(2·5)
5	10	46·7	(11·8)	9	31·6	(6·7)	10	30·0	(5·2)	10	19·3	(3·7)	10	21·4	(5·8)
6	7	21·6	(5·2)	10	19·3	(3·1)	8	17·6	(3·3)	10	17·9	(4·5)	10	16·5	(3·1)
Average		40·9			41·1			32·5			22·5			24·8	

n = Number of determinations.

contrasts indicates a significant ($p < 0{\cdot}01$) linear decrease in shear test values with increasing dosage. These findings correlate well with the decrease in tensile strength measured on the raw muscle.

Sensory evaluation tests on cooked irradiated pork and beef (Bailey *et al.* 1964) and on heat enzyme-inactivated utility beef (Kauffman & Harlan, 1969) indicate that irradiation promoted a tenderizing effect. Both studies concluded that the tenderization was due to the solubilization of collagen. Bailey further postulated that irradiation of collagen results in the scission of peptide bonds leading to the formation of lower molecular weight fragments which, in turn, are more soluble.

Examination of the muscles by transmission electron microscopy showed no structural differences at the myofibril level between the control sample (Fig. 4(a)) and the sample irradiated at 50 kGy (Fig. 4(b)). The sarcomere length before and after treatment remained constant at 2·4 μm. The minor

TABLE 3
Effect of Irradiation on Warner–Bratzler Shear Test of Cooked LD Muscle

	Compression forces (kg)				
	Control	*5 kGy*	*10 kGy*	*30 kGy*	*50 kGy*
n	9	9	8	9	10
Mean	10·01	8·0	9·84	6·01	5·76
SD	2·37	2·48	2·28	1·59	1·68

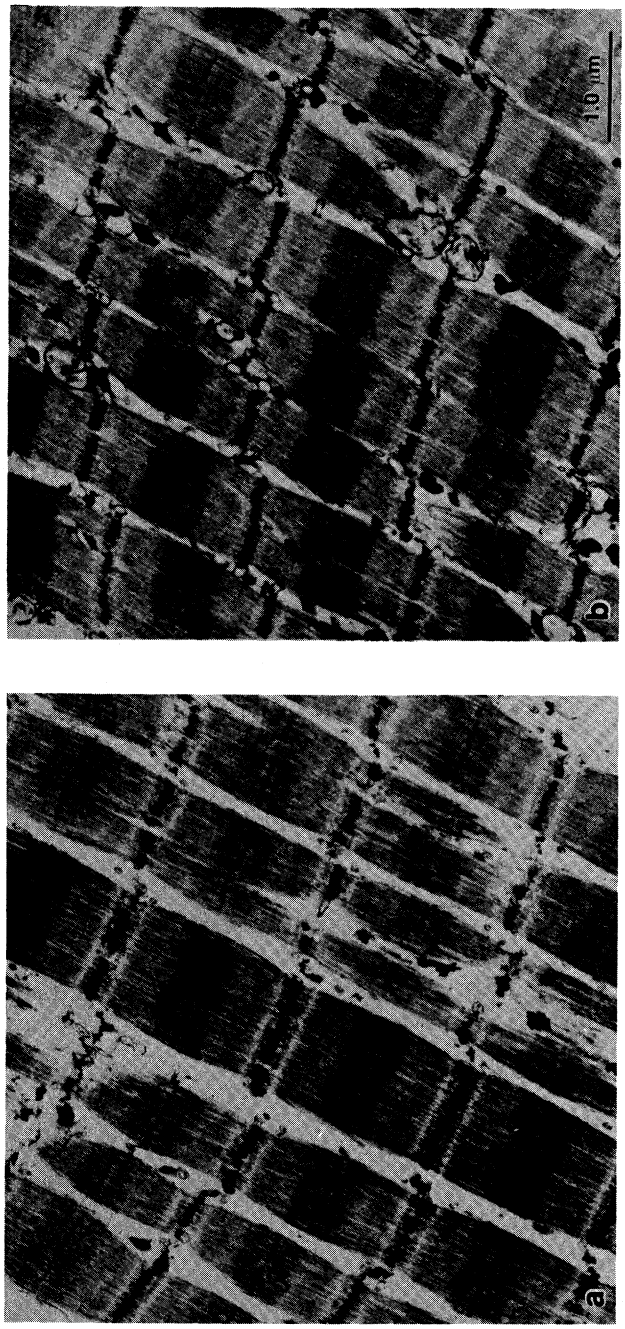

Fig. 4. Electron micrograph of *Longissimus dorsi*: (a) control; (b) 50 kGy.

variations in staining intensities within the sarcomere are attributed to the varying thickness of the myofibrils contained within the ultra-thin section. No changes were found in the perimysial or endomysial connective tissue. The effect of irradiation at levels up to, and including, 50 kGy therefore caused no observable changes in the myofibril structure of the muscle tissue. This does not preclude the possibility that irradiation does induce some change in structure; however, they are not readily apparent.

CONCLUSION

The physical state of a sample (dry, moist, frozen solid), presence or absence of free radical scavengers, and other conditions (temperature/oxygen) are factors which can mediate or intensify the effects due to ionizing radiation. Under the conditions of this study irradiation of raw LD muscle at sterilizing doses of 30 kGy and above at 0°–4°C led to apparent progressive increases in the fragmentation of myofibrils, a significant decrease in the tensile strength of raw muscle and a decrease in myosin. There was also a decrease in the force required to shear cooked muscle.

Changes induced by low levels of ionizing radiation (up to 10 kGy) were subtle and caused no major discernible effects on the textural characteristics of fresh or cooked muscle, when physical testing procedures, gel electrophoresis or transmission electron microscopy were employed. These results further indicate that the irradiation of raw meat up to a dose of 10 kGy, the maximum dose approved by WHO, would be difficult to detect when examined by such physical techniques.

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